Effects of Simultaneous Low-level Dietary Supplementation with Inorganic and Organic Selenium on Whole-body, Blood, and Organ Levels of Toxic Metals in Mice

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Classical experiments have demonstrated that Se compounds protect against the toxicity of several toxic metals in acute experiments with simultaneous parenteral administration of high doses of Se and the toxic metal. Blood and organ levels of the toxic metals were increased, conceivably due to formation of inert Se complexes. Less is known about effects of long-term Se status on the toxicokinetics of toxic metals. Possible Se interactions in toxic metal biokinetics should therefore be studied at Se levels ranging from those just sufficient to avoid Se deficiency and up to those believed to be optimum in relation to antioxidative and other beneficial effects of Se. The toxic-metal exposure levels investigated should be similar to those occuring in human populations that are not occupationally exposed. To study interactions between Se and toxic metals at ultralow exposure levels, mice were fed semisynthetic diets containing different levels of Se. The mice were given ultralow doses of metal salts either as a single oral dose by stomach tube or as prolonged exposure in the drinking water. Diets with high or normal Se levels slightly, but nonsignificantly increased the whole-body retention (WBR) of Hg^{++} and CH_3Hg^+ compared to a diet low in Se. The dietary Se level was, however, without effect on the WBR of Cd^{2+} and Ag^{2+} in single-dose experiments. During prolonged exposure, the diets fortified with Se increased the WBR of Ag^{2+}, had no effect on WBR of Hg^{2+}, and reduced the WBR of CH_3Hg^+ and Cd^{2+}. During prolonged exposure, the diets fortified with Se reduced blood Hg^{++} while organ levels were unaltered. Blood and organ levels of CH_3Hg^+ were reduced or unaltered. Diets with added Se reduced blood and organ levels of Cd^{++} but increased blood and organ levels of Ag^{++}. The blood lead level was reduced by Se supplementation. These results are in contrast to those previously published for Se effects on the toxicokinetics of Cd and Hg compounds. The results indicate, that Se supplementation might be beneficial in populations exposed for extended periods to increased environmental levels of certain toxic metals, e.g., Cd, Hg and CH_3Hg. — Environ Health Perspect 102(Suppl 3):321–324 (1994).

Key words: diet, selenium, interactions, biokinetics, cadmium, inorganic mercury, organic mercury, silver

Introduction

Classical animal experiments have demonstrated that Se compounds may protect against the toxicity of several toxic metals — As, Cd, Pb, and inorganic and organic Hg. These experiments were mainly acute with simultaneous parenteral administration of high doses of Se and toxic metal; blood and organ levels of the toxic metals were found to be increased, conceivably due to formation of inert Se complexes. For reference to the original literature, see the extensive review by Nordberg et al. (*1*). The presently available knowledge about interactions between inorganic (i) and organic (o) species of Se and toxic metals in acute exposure experiments is briefly summarized in Table 1.

Less is known about effects of long-term Se status on the toxicokinetics of toxic metals. Possible Se interactions in toxic metal biokinetics should therefore be studied at Se levels ranging from those just sufficient to avoid Se deficiency to those believed to be optimum in relation to antioxidative and other beneficial effects of Se. The toxic-metal exposure levels investigated should be similar to those occuring in human populations that are not occupationally exposed.

The present study was aimed at investigating effects of nutritional Se status (i-Se and o-Se) on the toxicokinetics of toxic metals administered to mice at dose levels relevant for human exposures. Mice fed semisynthetic diets containing different levels of Se were given ultralow doses of metal salts labeled with γ-emitting isotopes. The optimum natural dietary source for Se is probably selenomethionine (SEM), as this methionine analog is absorbed almost completely, while selenite is absorbed

Table 1. Reported effects of Se compounds on the toxicokinetics of toxic metals.[a]

Metal	Effect
As	i-Se decreases i-As toxicity
Cd	i-Se decreases Cd toxicity; blood and organ Cd levels increase; Cd:Se = 1:1, Se is probably selenide
Pb	Dietary i-Se decreases subchronic i-Pb toxicity; organ Pb and Se levels increase after combined exposure
i-Hg	i-Se decreases i-Hg toxicity; blood and organ Hg levels increase; Hg:Se = 1:1, Se is probably selenide. o-Se decreases i-Hg toxicity less efficiently than does i-Se
o-Hg	i-Se decreases o-Hg toxicity less efficiently than it decreases i-Hg toxicity. o-Se only marginally affects o-Hg toxicity

[a]The data were obtained mainly in acute experiments in rats exposed simultaneously to Se and toxic metals by parenteral routes. For reference to original publications, see Nordberg et al. (*1*).

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Table 2. Metal compounds, γ-emitting isotopes and dose levels used in the experiments.[a]

Isotope	$t^1/_2$, days	γ-lines, keV	Dosage	Single dose, µg/kg	Drinking water, ng/l
^{109}Cd	453 d	22, 88	$CdCl_2$	1	6
^{203}Hg	47 d	279	$HgCl_2$	1	6
			CH_3HgCl	1	6
^{110m}Ag	253 d	658	$AgNO_3$	10	60
–			$PbCH_3COO$	–	60

[a] For lead, a γ-emitting isotope was not available to us.

about 50%. Certain natural Se species apparently have a very low bioavailability. Accordingly, the low-Se diet contained only SEM, and a mixture of SEM and selenite contributing identical amounts of Se was added to the two Se fortified diets as specified below.

Materials and Methods

A total of 324 male Bom:NMRI mice were fed modifications of a standard semisynthetic diet used in our laboratory. This diet contains dietary fibers (7% w/w Whatmann cellulose) and all micronutrients. The energy contribution in the diet was: protein (caseinate) = 20%, lipid (coconut:soy 2:1) = 40%, carbohydrate mix = 40%. The Se content in ordinary mouse pellets used in our animal facility is specified by the producer as 900 µg Se/kg. Se in the standard semisynthetic diet is 150 µg Na_2SeO_3/kg corresponding to 50 µg Se/kg. For this experiment, three diets were prepared with varying Se contents: 12,5 µg/kg Se = 31,25 µg/kg SEM (low Se); 62.5 µg/kg Se = 99.25 µg/kg SEM and 68 µg/kg selenite (normal Se); or 112,5 µg/kg Se = 167.25 µg/kg SEM and 136 µg/kg selenite (high Se). Groups of 12 mice were fed these diets for at least three weeks before exposure to a metal salt labeled with a γ-emitting isotope as specified below. For single-dose exposure, the mice received a single oral dose of an aqueous solution of the γ-labeled metal salt by stomach tube. For drinking-water exposure, the γ-labeled metal salt was added to the drinking water. The concentrations of γ-labeled metal salts in the gavage solution and in drinking water were chosen as low as allowed by the specific activity of the commercially available isotopes and the detection limit in the γ-counter used for organ counting. For lead, a γ-emitting isotope was not available to us; accordingly only a drinking water experiment was carried out. The lead concentration in the drinking water was chosen according to the detection limit for AAS determination of blood lead. The metal compounds and isotopes, and the dose levels used, are specified in Table 2.

Toxicokinetic Analysis

All animals were counted in a whole-body counter (NaI well crystal Ø = 50 mm, 125 mm deep attached to a Searle 1195 R γ-counter) immediately after single-dose exposure, or at day 1 after starting the drinking-water exposure, then regularly for 14 days. After sacrifice, blood (drinking-water exposure) and organ concentrations were determined in the Searle 1195 R γ-counter. The detection limit was calculated for each isotope as the mean background value + 3 standard deviations based on 40 background countings. Blood lead levels were measured by graphite furnace AAS with Zeeman correction. Results are presented as medians and statistically

Table 3. Effects of dietary Se levels on WBR (% of initial dose in single dose experiments, program metal in drinking water experiments) and organ distribution (pg/organ, pg/g blood) of metals after single dose or drinking water exposure.

Se diet:	Low		Normal		High		Low		Normal		High	
Experiment	Single dose	Drinking water	Single dose	Drinking water	Single dose	Drinking water	Single dose	Drinking water	Single dose	Drinking water	Single dose	Drinking water
Metal compound	$HgCl_2$						CH_3HgCl					
WBR	2.2	27.5	3.1	18.5	2.9	24.5	48	93	52	64*[c]	55	66
Liver	54	6.05	80	4.89	83	6.36	1 838	6.89	1659	4.61*[c]	2234	6.44
Kidneys	263	20.2	421*[b]	17.3	345	28.19*[b]	1 625	9.20	1873	8.23	1835	7.14
Lungs	–[b]	–[b]	–[b]	–[a]	–[a]	–[a]	205	1.35	231	1.17	231	1.01*[c]
Brain	–[b]	0.82	–[a]	0.60	–[a]	0.84	177	0.85	219	0.49*[c]	216	0.79[d]
Blood	–[b]	0.40	–[a]	0.28	–[a]	0.32	550	1.11	650	0.70*[c]	750	1.19[d]
Carcass	134	7.40	195	4.93	202	6.25	14 520	62.7	17320*[c]	38.5*[c]	14°000[d]	41.6*[c]
Metal compound	$CdCl_2$						$AgNO_3$					
WBR	0.6	14.4	0.8	6.4*[c]	0.6	6.4	0.011	83	0.009	108	0.12	158
Liver	64.1	0.70	66.9	0.48	63.1	0.46	–[b]	0.42	–[b]	0.78	–[b]	1.03
Kidneys	89.7	0.72	92.3	0.52	68.8	0.53	110	0.048	73	0.062	100	0.092
Lungs	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]
Brain	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	100	0.43	87	0.57	127	0.64*[c]
Blood	–[b]	0.003	–[b]	0.003	–[b]	0.002	–[b]	0.22	–[b]	0.45	–[b]	0.44
Carcass	14.3	1.084	18.1	0.57*	15.5	0.67	–[b]	6.95	–[b]	6.00	–[b]	6.16

[a] Medians, $n = 12$. [b] All or some of the data in the experimental series were below the detection limit. [c] $p < 0.05$ compared to low Se diet in the same experiment. [d] $p < 0.05$ compared to normal Se diet in the same experiment.

Table 4. Summary of results.

i-Hg	Single dose	Se increases WBR by ~ 50% by increasing systemic deposition/retention
	Drinking water	Se slightly and nonsignificantly reduces WBR, high Se increase kidney deposition
o-Hg	Single dose	Se increases WBR slightly, possibly by increasing uptake and/or reducing excretion
	Drinking water	Se reduces WBR by ~ 50% by reducing systemic deposition/retention
Cd	Single dose	Se insignificantly affects WBR and organ distribution
	Drinking water	Se reduces WBR by reducing systemic deposition/retention
Ag	Single dose	Se insignificantly affects WBR and organ distribution
	Drinking water	Se increases WBR by increasing systemic deposition/retention
Pb	Drinking water	Se slightly and non significantly reduces blood Pb

compared using the nonparametric Mann-Witney U-test.

Results

Whole-body Retention

Compared to the whole-body retention (WBR) in animals eating the diet low in Se, the diets with normal or high Se content slightly, but nonsignificantly, increased the WBR of Hg^{++} and CH_3Hg^+ but were without effect on the WBR of Cd^{2+} and Ag^{2+} in single-dose experiments. During prolonged exposure to metal salts in the drinking water, the diets fortified with Se increased the WBR of Ag^{2+}, was without a certain effect on WBR of Hg^{2+}, and reduced, in some cases significantly, the WBR of CH_3Hg^+ and Cd^{2+}, compared to the low-Se diet (Table 3).

Organ Distribution

In the single-dose experiment, the diets with normal or high levels of Se seemed to enhance the deposition of i-Hg in liver, kidneys, and carcass compared to the low-Se diet. However, in the drinking-water experiment, unchanged or reduced organ deposition of i-Hg was noted, except in kidneys, where animals eating the high-Se diet had significantly increased deposition. This could signify enhanced excretion. During this prolonged exposure, normal or high Se diets reduced blood Hg^{++}. Similarly, organ levels of Hg tended to be increased in animals eating the diets with normal or high-Se levels after single dose exposure to o-Hg, while after drinking-water exposure to o-Hg, organ levels of Hg were most often reduced in animals eating diets with normal or high Se levels. The blood levels of CH_3Hg^+ were reduced or unaltered (Table 3).

After a single-dose exposure to cadmium, organ levels of that metal were in most cases similar in the groups eating the three different Se diets. In the drinking-water experiment, however, Cd in organs and blood was in most instances reduced in the groups eating the fortified diets. The blood and organ levels of Ag were extensively increased by normal or high dietary Se levels after drinking water exposure (Table 3).

In the drinking-water experiment with lead, the blood levels of lead in the two groups (n = 12) eating diets with normal or high Se levels were 6.2 (4.1–7.3) and 6.2 (4.1–8.3) ng/g (25 and 75 percentiles) as compared to 8.3 (6.2–17.6) ng/g in the group eating the low-Se diet, indicating a nonsignificant reduction in blood lead by selenium.

Discussion

The results presented here are preliminary. To evaluate metal exposure levels that are relevant compared to normal or slightly increased human exposure levels, the experimental model employed metal doses at the borderline of the detection limits. As a result, many of the data sets obtained contain large variations both within and between groups exposed to the same toxic metal and different dietary Se levels. Although several rather large differences are not statistically significant, the combined results show a clear trend. The results are summarized in Table 4.

Drinking-water exposure is more relevant for human dietary metal exposure than single-dose exposures. In most of the drinking-water experiments reported here (Ag is an important exception), the result of increasing the dietary Se content by adding both i-Se and o-Se was a reduction of WBR and organ deposition of the toxic metals. The single-dose experiments gave results that are more in agreement with those from the classic investigations in rodents mentioned in the introduction. Thus, simultaneous parenteral administration of selenite and $HgCl_2$ resulted in extensively enhanced body retention of mercury, reduced urinary excretion, and enhanced organ deposititon (*2–7*). Also, the protective effect of selenium compounds on organ toxicity of cadmium in acute exposure experiments is accompanied by enhanced cadmium deposition in these organs (*1,3,8,9*).

In conclusion, the results presented here indicate that effects of selenium compounds on the toxicokinetics of Cd and Hg compounds in human exposure would probably be different from effects predicted from previously published experimental animal studies, because Se reduced rather than increased toxic metal retention and organ deposition in exposure situations relevant for human exposure. The small effect of Se exposure on Hg kinetics observed in the present study is in accord with other recent results from our laboratory (*10,11*). As the present investigations used exposure levels for Se and toxic metals relevant for normal or slightly increased human exposure, the results indicate that Se supplementation might be beneficial in populations exposed to high environmental levels of toxic metals.

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